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BIOCOAGULATION/BIOFLOCCULATION - A LITERATURE REVIEW

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BIOCOAGULATION/BIOFLOCCULATION - A LITERATURE REVIEW

by

D.W. Koren*

ABSTRACT

This review will provide some general background information concerning coagulation/flocculation as well as some of the theories that have been proposed to explain these processes. A discussion of the nature of biopolymers and the factors affecting their performance is included. A listing of various microbes and biopolymers extracted from them that could be used as coagulating/flocculating agents will also be provided. Some synthetic polymers that are currently used are described.

Keywords: coagulation, flocculation, bioflocculation

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INTRODUCTION

Waste water contains a number of substances, these may be divided into three categories (Degremont, 1991):

- suspended solids (ss); these may be mineral in origin (sand, silt, clay), organic (products from the decomposition of plant and animal matter) and microorganisms (bacteria and algae, etc.);
- colloidal substances (less than 1 micron); these may be of the same origin as described above; and
- dissolved substances (less than several nanometres); these include cations and anions, dissolved organic matter and gasses (O_2, CO_2, H_2S, etc.).

The purpose of coagulation and flocculation processes is to facilitate the removal of suspended solids and colloids by destabilizing them. There is evidence that some dissolved species are also removed in these treatment processes.

Coagulation is generally considered a chemical or physical process in which the charge on the colloidal material is neutralized by an oppositely charged chemical, permitting them to collide to form "microflocs". In the case of negatively charged particles cationic coagulants are used, these may be inorganic (e.g., alum and iron) or inorganic or organic polymers with cationic functional groups. The primary function of these is to neutralize the negative charge on the particles. Coagulation is the first step in the clarification process because the "microflocs" that are formed are still virtually invisible and too small to settle in a sedimentation pond or thickener. Stirring of the solution is necessary to cause collisions of the "microflocs", eventually larger flocs are formed. This growth in size is termed flocculation and is usually aided by the addition of flocculating agents (e.g., polyacrylamide). The primary function of flocculants is to join several "microflocs" by providing a bridge between the neutralized particles. Flocculation results in a considerable increase in the size

and density of coagulated flocs, resulting in a faster settling rate. Flocculants are often ionic but their function is not to neutralize the particles; the charge is primarily an indication of how it will uncoil in a given situation.

In the past, metal coagulants (i.e., alum and iron salts) have been most often used for water clarification. When added to water in the pH range of 6-7, they form positively charged species which are capable of neutralizing negatively charged particles. These coagulants are highly pH sensitive, therefore if the pH is not in the proper range clarification is often poor. Activated silica was introduced in the 1940's, it was found to improve the performance of the alum and iron salts. In the 1950's a wide range of synthetic flocculants, known as polyelectrolytes were introduced into water treatment processes. Polyelectrolytes are large water-soluble organic molecules which incorporate ion exchange sites, giving the molecules an ionic charge. Cationic polyelectrolytes are designed to work in acidic solutions while anionics work well in alkaline solutions. In recent years, the synthetic polymers have to a large extent replaced the inorganic salts as coagulants/flocculants. The main advantages of these polymers are (Allied Colloids, 1978):

- increased cost efficiency
- increased technical efficiency
- reduced handling problems
- reduced scaling/corrosion problems
- effective over a much wider pH range
- considerably reduced sludge volumes.

THEORY

Colloidal and Surface Chemistry

Coagulation/flocculation of particles would be spontaneous if it were not for the forces that result in stabilizing the suspensions. All particles possess attractive forces, known as London-Van der Waals forces, which act upon each other at short distances of separation. When particles are close together these forces predominate and natural coagulation of the

particles will occur. However, suspensions are often stabilized by repulsion forces which prevent the particles from coming close enough for these attractive forces to predominate (Allied Colloids, 1978). There are two sources of these repulsion forces: the adsorption of water by the particles form solvation layers which repel each other and the acquisition of electrical charge on the suspended solid surface. These forces of repulsion not only prevent the particles from coming together but hinder settling by keeping the particles in constant motion.

Electrical charge on particle surfaces may be acquired by two methods. In the first, high valence cations such as Al^{3+} and Si^{4+} in the clay lattice structure may be substituted by lower valence cations such as Mg^{2+}, Zn^{2+}, Na^{+} and Li^{+} which results in a positive charge deficiency (Lanza et al., 1982). The second method is by preferential adsorption of ions from the solution; adsorption occurs at specific sites which are formed as a result of the exposure of inner lattice atoms or lattice atoms at clay particle surfaces (Black et al., 1966).

Due to the surface charge that the particles have acquired, ions of the opposite charge will be attracted to them, forming a layer of bound ions. When a particle moves, the layer of bound ions moves with the particle. The potential that exists between the layer of these bound counterions and the bulk solution is the Zeta Potential. The Zeta Potential is often used as a measure of the degree of repulsion that exists between particles and will thus determine how stable a suspension will be. Generally, the higher the Zeta Potential, the more stable is the suspension.

Coagulation/Flocculation Mechanisms

To reduce the forces keeping the suspended particles apart it is necessary to reduce the charge between particles. Charge can be reduced by the addition of reagents which possess an opposite charge to that carried by the particles. For negatively charged particles, Al^{3+} and Fe^{3+} salts are mainly used. Most pronounced coagulation occurs when the Zeta Potential has been reduced to near zero but not necessarily zero. The reason for this is that

it is only necessary to reduce the potential to a level which will permit interparticle attractive forces to dominate. Optimum doses are required since excess positive charges can be built up. The flocs produced are weak and can be broken down under high shear (Moss and Dymond, 1978), their stability can be improved by the addition of flocculants which will effectively form a bridge between several flocs.

Flocculants are generally natural or synthetic water-soluble organic polymers of high molecular weight (Moss and Dymond, 1978). Polymers of high molecular weight are long enough to adsorb one particle at one end and a second particle at the other end; even higher molecular weight polymers are capable of adsorbing several particles at once, forming a three dimensional matrix (Allied Colloids, 1978).

Over the years there have been a number of theories hypothesized to explain the function of flocculating agents in the aggregation of suspended solids. The theory that is generally accepted today has been put forward by Tenney and Stumm (1965) and explains the phenomenon in terms of polyelectrolyte interaction (Brierley and Lanza, 1982). In water, functional groups on the polyelectrolyte dissociate to produce charged molecules. The repulsion of similarly charged functional groups along the length of the polyelectrolyte chain causes a stretched configuration and results in a polyelectrolyte solution with a high viscosity (Brierley and Lanza, 1982). When it is introduced into a solution containing charged colloidal particles a diffuse layer of colloidal material is adsorbed around the charged functional groups. The adsorption of the polyelectrolyte onto several hydrophobic colloids at the same time is referred to as bridging. In some cases it is necessary to add salts to the suspension, the salts reduce the repulsion of the electrical double layer and also enhance adsorption of the polyelectrolyte. As the repulsion between the functional groups diminishes, the polyelectrolyte assumes a coiled configuration (Brierley and Lanza, 1982). Under static conditions the polyelectrolyte will precipitate, carrying with it the adsorbed colloidal material.

Other mechanisms that have been put forth to explain the aggregation of particles are charge effect neutralisation, hydrophobic interaction and mutual dehydration (Unz, 1987). Charge effect neutralisation involves the compression of the diffuse electrical double layer through the introduction of electrolyte ions; this effectively reduces the energy of the particle and permits short range attractive forces to prevail. Hydrophobic attraction occurs when predominantly non-polar particles in an aqueous phase are brought together due to a thermodynamic favourable reduction in the entropy when the polar water molecules surrounding the particles are displaced into the bulk solution. The presence of ions such as NaCl and Na_2SO_4 reduces the water's ability for particle solvation, this in turn increases the extent of the hydrophobic interaction. Mutual dehydration refers to phase separation of hydrated (hydrophillic) colloids by non-ionic polymers resulting in an insoluble product and the release of water molecules from the reactants (Walles, 1968).

The nature of the adsorption forces involved in the action of polymeric flocculants is not very well understood. The following forces though have identified as playing a role in the process: electrostatic, hydrogen bonding, chemical bonding and hydrophobic interactions (Kitchener, 1972). With cationic polymers, adsorption on negatively charged particles occur due to electrostatic interactions. Non-ionic polymers such as starches usually adsorb solids through hydrogen bonding of the hydroxyl groups on the polymer. Chemical interactions between functional groups of the polymer and metallic ions on the solid surface may lead to the formation of salt-type compounds as in the case of polyacrylate and the Ca^{2+} ion present in kaolin (Michaels and Morelos, 1955). Adsorption may also be caused by the association of non-polar groups of polymers with hydrophobic surfaces; examples of this is the adsorption of dextran onto oxidised coal (Miller et al., 1983) and molybdenite (Mackenzie, 1980).

Factors which affect flocculation are: polymer concentration, polymer configuration, particle concentration, agitation and the solution's pH and ionic properties. It has been observed that as the polymer concentration is increased, the turbidity of the supernatant solution decreases; once the particle surface becomes saturated the turbidity increases. The

optimal concentration is a function of the surface area of the colloidal particle. Adsorption of a polymer to a solid surface is generally not a function of its molecular weight but rather its chain length (Harris and Mitchell, 1973). Another important factor is the agitation. Polymer adsorption is very rapid but the rate controlling step in the flocculation process is usually the interparticle collision rate, increasing the agitation results in an increased collision rate (Hannah et al., 1967).

BIOPOLYMERS

Biopolymers of microbial origin include polysaccharides, mucoproteins, polypeptides and nucleic acids (Harris and Mitchell, 1973). These can generally function as flocculants due to their relatively large molecular weight and low charge density. Some polymers that are produced are slightly ionic or can be rendered ionic by chemical treatment and thus may be capable of neutralizing some of the charge on the particles and act as coagulants.

Since polysaccharides are composed primarily of neutral sugars, flocculation may be due hydrogen bonding; whereas extracellular polymers made of other substances (e.g., proteins, etc.) may flocculate colloids as a result of ionic bonding. It has been shown that the extracellular microbial polymers also act as agents for the relatively non-specific adsorption of dissolved or suspended organic, particulate and ionic substances (particularly metals) (Dugan, 1970a; 1970b; Dugan et al., 1970). Metal binding sites have been identified on neutral and anionic polysaccharides (Steiner et al., 1976) and proteins fractionated from sewage sludges (Boyd et al., 1979).

Polysaccharides

Several types of microorganisms have been identified as producing polysaccharides which aid in coagulation/flocculation processes; these can be divided into categories based on the type of polysaccharide they produce (Easson et al., 1987). First there are the cellulose producing bacteria (e.g., species of Pseudomonas, Aerobacter, etc.); for these microbes,

flocculation occurs when the cells become embedded in a network of polysaccharide fibrils. Other floc forming bacteria form capsular polysaccharides enclosing large packets of cells (e.g., Zoogloea ramigera 115). Yet others produce water-soluble ionic exopolysaccharides that cause flocculation to occur in a manner analogous to synthetic polyelectrolyte flocculants.

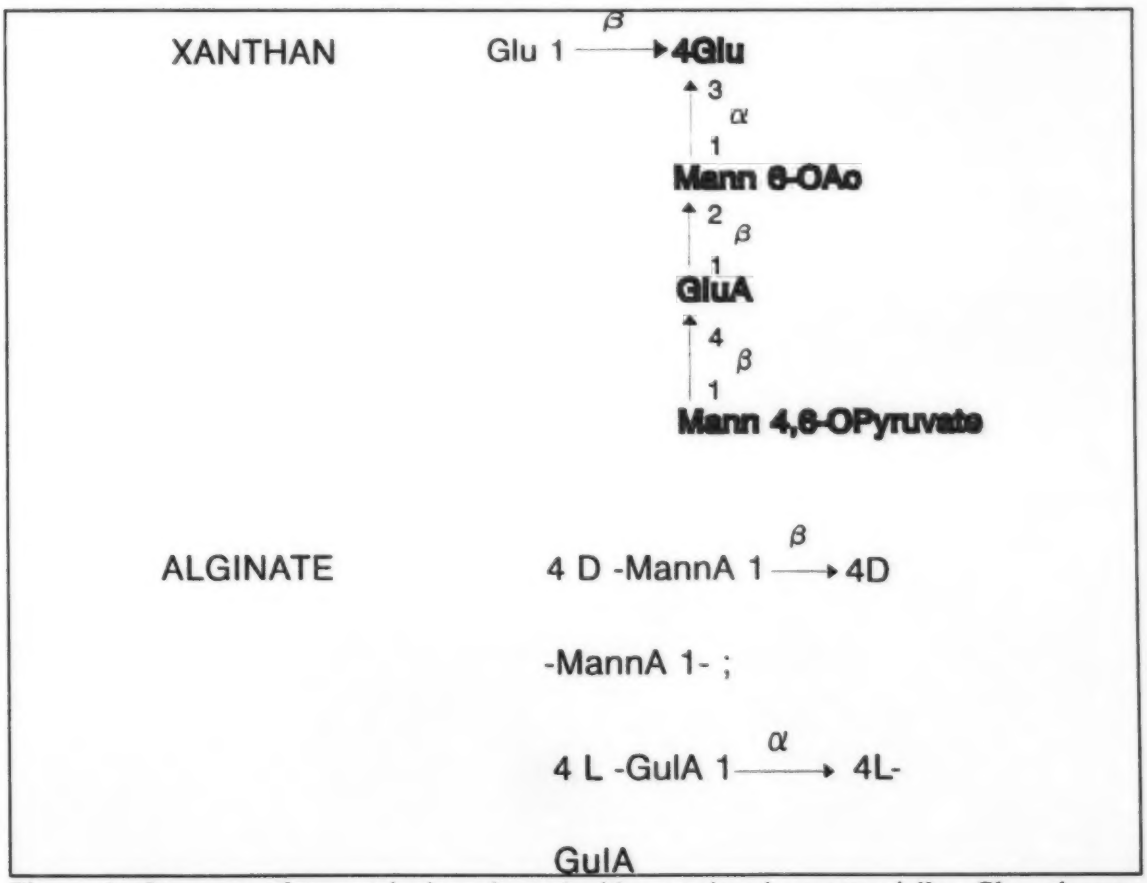

Figure 1: Structure of two anionic polysaccharides produced commercially. Glu=glucose; GluA=glucuronic acid; GulA=guluronic acid; Mann=mannose; ManA=mauronic acid; Ac=acetate.

Some polysaccharides that are produced commercially by microbial processes are: xanthan, dextran, scleroglucan, pullulan and curdlan (Crueger and Crueger, 1984). Alginate is another polysaccharide that is produced in large quantities, it is extracted from seaweed but in the future it is expected to be produced by microorganisms. Xanthan and alginate are anionic polysaccharides since they are composed of uronic acid residues (Figure 1), the others are nonionic in character. Xanthan, produced from Xanthomonas species; the basic repeating unit of xanthan consists of a pentasaccharide containing glucose, mannose, glucuronic acid, acetate and pyruvate (Crueger and Crueger, 1984). This polymer is stable in most salts but in the presence of polyvalent metals will precipitate at high pH values (Kang and Cottrell, 1979). Dextran is produced by Leuconostoc species, it is composed of glucose units with 1,6 glycosidic bonds, some 1,2-, 1,3- or 1,4-glycosidic bonded units are also present (Crueger and Crueger, 1984). Molecular weights range from 15,000 to 500,000. Dextran has no charged groups and is compatible with salts, acids and bases (Kang and Cottrell, 1979; Brierley and Lanza, 1982). The microbe Sclerotium, produces a polysaccharide that has been termed scleroglucan, which is a linear D-glucan connected primarily with beta-1,3-glycosidic bonds and occasionally beta-1,6 bonds (Crueger and Crueger, 1984). Some properties of note are its high viscosity and its stability at high temperatures over a broad pH range and in the presence of electrolytes (Sutherland, 1983). Another fungal polymer recognized as being of commercial interest is pullulan, it is an extracellular polysaccharide that is produced by the fungus Aureobasidium (Pullulans) pullulans (Ueda et al., 1963). This polymer is made of glucose with alpha-1,4- and a few alpha-1,6 glycosidic bonds. Industrially pullulan has been used in the flocculation of fine clays produced from hydrometallurgical processes (Kang and Cottrell, 1979). It has also been patented for the flocculation of clay slimes produced in the beneficiation of uranium, potash and aluminum (Zajik, 1967). Pseudomonas aeruginosa and Azotobacter vinelandii produce alginate which is a copolymer of D-mannuronic acid and L-glucuronic acid. Alcaligenes faecalis var. myxogenes produce curdlan and succinoglucan. Succinoglucan contains glucose, about 10% succinic acid and galactose, curdlan is a polymer of glucose units joined by beta-1,3 glycosidic bonds (Crueger and Crueger, 1984).

Phosphomannan is composed of mannose and mannose-6-PO_4, it is produced by the yeast Hansenula holstii (NRRL Y2448/ATCC 12837) (Goren, 1968; Smiley, 1976). Arthrobacter viscosus (NRRL B1973) produces a polymer which is composed of equal portions of glucose, galactose and manuronic acid. This polymer was shown to be stable over the pH range 5 to 10 and at high temperatures. A black pigmented extracellular polysaccharide is produced by the yeast strain NRRL Y6272 (Sanford et al., 1975). This polymer is made of N-acetyl-D-glucosaminuronic acid and N-acetyl-D-glucosamine, it is thought to have potential for industrial use since it contains both acidic and basic groups and disperses readily to produce a viscous solution (Brierley, 1981). A group of floc forming bacteria are indigenous to aerobic waste treatment facilities, these organisms produce exocellular fibrillar polymers which appear to play a major role in the flocculation part of the activated sludge process (Friedman et al., 1969). Strains of Zoogloea ramigera are common inhabitants of activated sludge reactors. Isolate I-16-M produces a fibrillar cellulose-like exopolysaccharide (Dugan, 1975), while strain 115 makes a water soluble capsular polysaccharide composed of glucose, galactose and pyruvate (Dugan, 1975; Ikeda et al., 1982). In addition to its flocculating ability, Z. ramigera 115 flocs are capable of absorbing 40% of their total weight in heavy metals (Norberg and Persson, 1984).

Other microbes that produce exopolysaccharides are listed in Table 1.

Other Polymers

A number of other polymers produced by microorganisms may also play an important role in the flocculation process, these are nucelic acids and proteins. Bomstein (1972) described the alkaline treatment of microbial nucleoprotein; the treated proteins were used for the flocculation of organic and inorganic wastes. Nucleoproteins were also produced by strains of Polyangium, Mysococcus, Sorangium, Flavobacterium, Micrococcus and Alcaligenes. Other microbes which were found to produce sticky extracellular DNA under

Table 1 - Microbes that Produce Extracellular Products which are Capable of Acting as Flocculating or Adsorbing Agents

Source of Extracellular Polymer	Extracellular Product	Ref.
Xanthomonas	Xanthan (glucose/mannose/glucuronic acid)	
sp.	(3/3/1)	7,8
campestris NRRL B1459	(2/1/1)	65
campestris (wild strain)		77
Leuconostoc	Dextran	
mesenteroides		7
sp.		4
Sclerotium glucanicum	Scleroglucan (D-glucan and D-glucopyranosyl)	8
Aureobasdium (Pullularia)	Pullulan (maltotriose or maltotetraose)	
pullulan		73
Hansenula	Phosphomannan (mannose, mannose-6-PO_4)	
holstii ATCC 12837(NRRL Y2448)		65
sp.		33
Arthrobacter	Polysaccharide (glucose/ galactose/mannuronic acid)	
viscosus NRRL B-1973	(1/1/1)	65
sp.		33
NRRL Y-6272 (yeast)	N-acetyl-D-glucosaminuronic acid/ N-acetyl-D-glucosamine	62
Zoogloea		
ramigera I-16-M	fibrillar cellulose like polysaccharide	32
ramigera 115		
filipendula	Polysaccharide (glucose/galactose/pyruvate)	
Cryptococcus sp.	Polysaccharide	33
Plectania sp.	Polysaccharide	33
Azobacter vinelandii ATCC 12837	Alginate	7
Beijerinckia indica ATCC 9037	Polysaccharide	7

Anabena sp.	Polysaccharides and other biopolymers	2
Chlamydomonas sp.	Polysaccharides and other biopolymers	2
Chlorella sp.	Polysaccharides and other biopolymers	2
Pseudomonas denitrificans	Cellulose and Polysaccharides	32
Corynebacterium sp.		80
Aspergillus sojae		51
Dematium sp.		63
Paecilomyces sp. I-1	Polysaccharide (galactosamine)	69
Rhodococcus erythropolis S-1		43
Polyangium sp.	Nucleoproteins	4
Mysococcus sp.	Nucleoproteins	4
Sorangium sp.	Nucleoproteins	4
Flavobacterium sp.	Nucleoproteins	4
Alcaligenes sp. faecalis var myxogenes	 Nucleoproteins Curdlan	 4 18
Micrococcus sp. halodenitrificans	 Nucleoproteins Sticky extracellular DNA	 4 36
Vibrio costicola	Sticky extracellular DNA	36
Pasteurella pestis (Yersinia pestis)	Sticky extracellular DNA	36
Klebsiella aergenes	Polysacharide proteins	12
Staphylococcus aureus	RNA, protein	36

nutrient deficient conditions were: Micrococcus halodenitrificans, Vibrio costicola and Pasteurella pestis (Brierley, 1981). Some other polymer producing microorganisms are listed in Table 1.

Factors Affecting Biopolymer Production

Several factors have been identified to affect the production of extracellular polymers in bacterial cultures and activated sludge: the ratios of carbon:nitrogen (polymer production has been shown to increase as the C:N ratio is increased), carbon:phosphorus, carbon:sulphur in the growth medium (Deguid and Wilkinson, 1953; Corpe, 1964; Wu, 1978); oxidation of extracellular polymers (Obayashi and Gaudy, 1973); dissolved oxygen concentration (Novak et al., 1977); biomass loading rates (Kiff, 1978); biochemical oxygen demand (BOD) (Coackley, 1969). It has also been found that microbial polymer production is increased when microorganisms are grown on carbohydrates rather than hydrocarbons and alcohols derived from petrochemicals (Slodki and Cadmus, 1978). Microbial polymer production is also strongly influenced by the growth phase of the microbe; generally the tendency for bioflocculation is greater under conditions of declining or endogenous growth (Busch and Stumm, 1968). It is thought that this may be due to the fact that while organisms are growing prolifically, new microbial surfaces are produced faster than surfaces can be covered with polymers. Newly formed cells therefore remain dispersed as long as they are growing rapidly, if growth ceases under conditions of long retention time in a biological reactor, the old cells adhere to each other forming bridges which leads to flocculation.

Since the functional properties of any biopolymer are dependent on its composition and structure, the ability to manipulate and control these characteristics will enable the engineering of more efficient bioflocculants, to this end, recombinant DNA technology has been applied with some success (Easson et al., 1987).

Polymer Extraction

Extraction and concentration of the polymers can be performed using physical and/or chemical methods. Samples of the culture are centrifuged at up to 33,000 g for 10-15 minutes and the soluble polymer present in the supernatant may be separated from the cell debris by decantation or filtration through a 0.2 micron filter (Lester et al., 1984). Ultrasonification may also be used to extract the polymers. Heat treatment, either boiling or autoclaving has also been used in the past. Chemical methods involve the treatment of a centrifuged pellet (2000 g for 20 minutes) with NaOH. The polymer is then extracted into the liquid and can be separated from the other cell debris by centrifugation. EDTA has also been used as an extractant (Brown and Lester, 1980). The polymers can be obtained in a dry form for storage or further analysis by precipitation in ethanol (Pavoni et al., 1972) or acetone (Lester et al., 1984). Some of polymers that have been extracted from microbial broth and the methods that were used are given in Table 2.

Table 2 - Polymers Extracted from Microbial Broth and used for their Flocculating Ability

Source	Product	Extraction Method	Ref.
Activated sludge	polysaccharide, protein, DNA, RNA	centrifuge, ethanol ppt.	58
Activated sludge	polysaccharide, protein	boiling, acid, EDTA, ultrasound, alkaline ext., ethanol and acetone ppt.	14
Activated sludge	polysaccharide, DNA, RNA	alkaline ext., etnanol ppt. and EDTA, ethanol ppt.	52
Activated sludge	polysaccharide, protein, DNA, RNA	ammonium hydroxide, blending, ethanol ppt.	71
Activated sludge	carbohydrate	centrifuge, ethanol ppt.	13
Activated sludge	hexoses, pentoses, hexosamines and methyl pentoses	colloid mill, ethanol ppt.	62
Activated sludge	glucose, pentose, glucuronic acid	ammonium hydroxide blending, ethanol ppt.	74

Activated sludge	galactose, fucose, mannose, glucuronic acid, glucose, xylose, rhamnose, ribose	boiling, ethanol ppt.	30
Activated sludge	glucose, mannose, rhamnose, galactose, maltose, lactose, hexosamine	boiling and alkaline extraction	76
Activated sludge	glucuronic acid neutral sugars	centrifugation, boiled, centrifuged, ethanol ppt, acetone dried	29
Z. ramigera NRRL B-3669M	glucose, galactose, mannose	boiling and alkaline extraction, ethanol ppt.	76
Z. ramigera 115	glucose	ultrasound, methanol ppt.	31
Z. ramigera 115	glucose, galactose	ammonium hydroxide, blending, ethanol ppt.	57
Z. ramigera MP6 and 106	glucosamine, D-methyl pentose amine, uronic acids	blending, CTAB ppt	28
Z. ramigera (isolated from activated sludge)	glucosamine, fucosamine	sodium hydroxide extraction, ethanol ppt.	72
Klebsiella sp.	D-glucose, galactose, L-fucose, mannose, a uronic acid	boiling and alkaline extraction	20
Aerobacter aeragenes A3 (51) (Klebsiella aerogenes)	D-glucose, L-fucose, glucuronic acid	sulphuric acid, acetone ppt.	61
Beijerinckia indica ATCC 9037 Leuconostoc mesenteroides Xanthomonas sp.	biopolymers	alcohol ppt.	7
Aspergillus sojae AJ7002	protein (25%) hexosamine (26%) 2-ketogluconic acid (32%)	centrifugation, NaOH dissolve, acetone ppt	51

BIOFLOCCULANTS

Work was carried out by Brierley et al. (1981) to test several microbiological processes for flocculation of finely divided clays generated from the mining and milling of phosphate. Polymers produced by Leuconstoc mesenteroides, Xanthomonas sp. and

Beijerinckia indica were isolated and found to flocculate dilute phosphatic clay slime at a rate comparable to polyethylene oxide; the supernatant after flocculation with the biopolymers though was more turbid. It was observed that when Cladosporium cladosporiodes spores were cultured in the clay solutions enriched with sugar and yeast extract they formed pellets encapsulating the clays thus yielding a clear supernatant. Kurane et al. (1986a) reported on the flocculating ability of Rhodococcus erythropolis. It was found that it could efficiently flocculate suspended solids such as Escherichia coli, yeast and kaolin clay. The addition of cationic ions such as Ca^{2+} were found to increase the flocculating activity significantly. Although the bacteria could produce the flocculant using a simple medium, some optimization was necessary for more efficient production (Kurane et al., 1986b).

Nakamura et al. (1976a, b) screened a number of microorganisms for their ability to flocculate baker's yeast. The polymeric substances in the culture broth of these microbes were precipitated by the addition of acetone; these precipitated substances were found to aid in the agglomeration of a number of microorganisms. Some species of microorganisms have a characteristic flocculant growth habit whereby the cells form clumps in the medium, these clumps are referred to as flocs. In the absence of agitation these flocs settle out and the growth medium becomes clear. A number of floc forming microorganisms have been described by Dugan (1987). Bioflocs of this type are referred to as Zoogloea and are capable of adsorbing several different cations including cobalt, copper and iron.

Flocculation has been identified as an important part of activated sludge systems (Brown and Lester, 1979). The activated sludge process can be divided into two unit operations: a biological reactor where large populations of microorganisms convert the dissolved or suspended solids to carbon dioxide, water and cellular material and a phase separator where the microorganisms form aggregates which settle out by sedimentation. The natural extracellular polymers in the sludge are all high molecular weight compounds (MW $>10,000$); the main groups of compounds produced are neutral and anionic polysaccharides (major constituents), proteins, RNA and DNA (Ryssov-Neilsen, 1975). The presence of

RNA and DNA may result from the lysis of the cells. These extracellular polymers are used, after extraction, as flocculants for inorganic, colloidal suspensions (Ryssov-Neilsen, 1975).

It has been observed that metals are removed from waste water in the activated sludge process, this is thought to be due to the adsorption of dissolved metals or fine particulates onto the sludge flocs (Oliver and Cosgrove, 1974; Brown et al., 1973). Brown and Lester (1979) summarized removal efficiencies of a variety of metals in activated sludge processes. From the results it appears that iron, copper, chromium and zinc have fairly high removal efficiencies while nickel, manganese, calcium and magnesium have lower removal efficiencies. Dugan and Pickrum (1972) demonstrated that the cell-floc matrix of Z. ramigera 115 will also adsorb a variety of ions from a mixed solution, the adsorption appears to be non-specific. Chen et al. (1974) have proposed a two stage process by which metals are removed in the activated sludge process. The first stage is a rapid uptake phase in which a large quantity of the metal is adsorbed by the cell floc. The second is a slow phase in which only small amounts are removed due to uptake into the actual metabolism of the microbe. Whereas, in the former case metal uptake is a physio-chemical process, in the latter it is a biological one.

CHEMICAL FLOCCULANTS

Modern water treatment facilities rely on the use of chemical agents to enhance the coagulation/flocculation of suspended solids. A listing of products available commercially can be found in Engineering and Mining Journal (Suthill, 1991).

The most commonly used inorganic coagulants used are those of iron and aluminum compounds. Some of those most often used are: alum $Al_2(SO_4)_3.14H_2O$; ferric chloride $FeCl_3.6H_2O$; ferric sulphate $Fe_2(SO_4)_2.3H_2O$; copperas $FeSO_4.7H_2O$ and sodium aluminate $Na_2Al_2O_4$. It was originally thought that the highly charged ions were responsible for the coagulation of suspended solids. Later, it was found that the hydrolysis products are

responsible for the destabilization of the solid particles (Gregory, 1989). Some products which contain polymerized forms of aluminum and iron are also available (e.g., polyaluminum chloride).

The majority of organic coagulants/flocculants are synthetic polyelectrolytes, natural products such as starches or alginates, or extracts from seeds (e.g., guar gums) are used but only for specific industrial applications (Suthill, 1991; Gregory, 1989). Coagulants are positively charged molecules of relatively low molecular weight (under 100,000); they are capable of forming bridges but are not as effective as flocculants (Kemmer, 1988). Flocculants are much higher molecular weight (between 10^6 and 10^7) enabling long bridges to be formed between flocs and thus enhancing particle growth. The flocculants are classified according to their functional groups: cationics, anionics and nonionics. The cationic polyelectrolytes are copolymers of acrylamide and a cationic monomer. The more common cationic flocculants are: dimethyl-amino ethyl-methacrylate (DMAEM), dimethyl-amino ethyl-acrylate (DMAEA) and diallyl-dimethyl ammonium chloride (DADMAC) (Figure 2). Anionic polymers generally incorporate a carboxyl group (-COOH) in their structure (Kemmer, 1988). Anionics are coppolymers of acrylic acid and acrylamide or hydrolyzed polyacrylamides (Figure 3). Nonionic flocculants used are polyacrylamides (Figure 4). Cationics are available as solutions, dry products and emulsions. Anionics and nonionics are produced in dry or emulsions form (Raman, 1981).

Synthetic flocculants used for mineral slurries tend to be anionic since these adsorb particles strongly, are of higher molecular weight than cationics and are less expensive (Moss and Dymond, 1978). Other types of synthetic polymers that are available for the flocculation of suspended solids include: polyvinyl alcohol, polyethylene oxide (non-ionic); sodium polystyrene sulphonate, copolymers with 2-acrylamido-methyl propane sulphonic acid (anionic); polyethyleneimine, polydiallyldi-methylammonium chloride, hydroxy-ionenes (cationic) (Gregory, 1989).

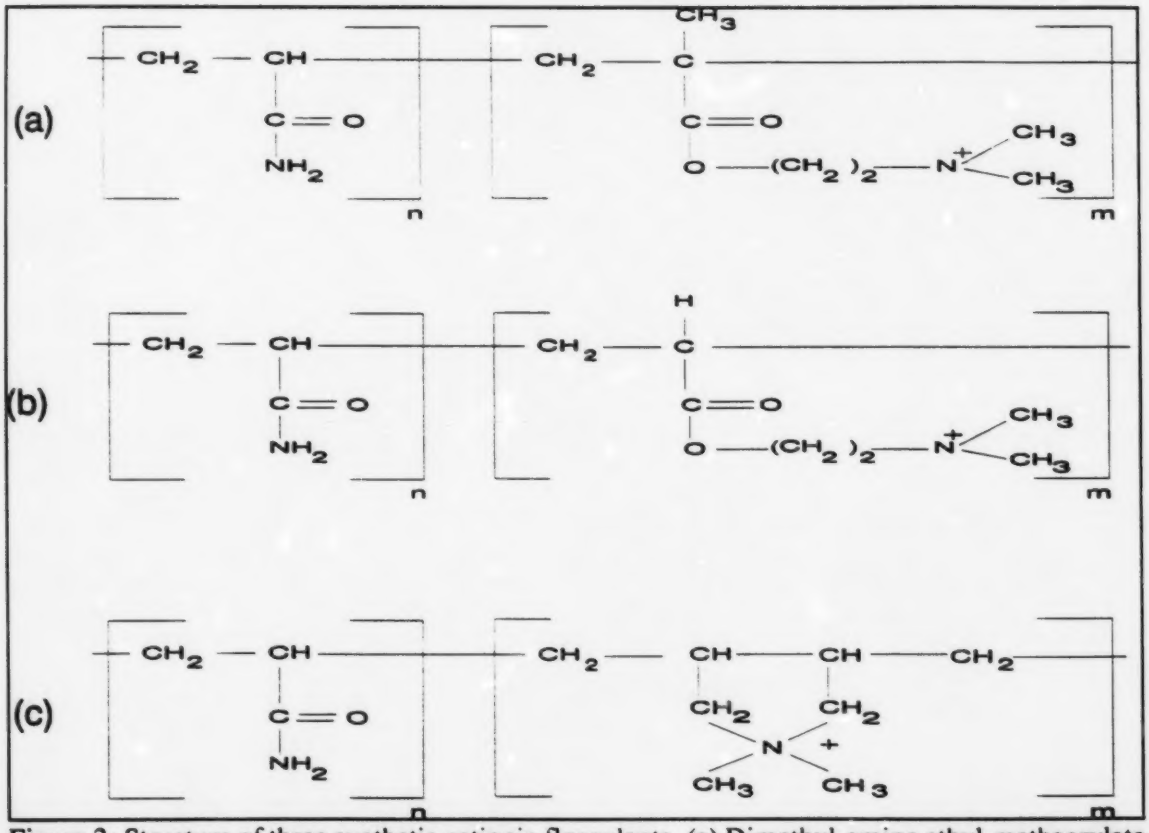

Figure 2: Structure of three synthetic cationic flocculants. (a) Dimethyl-amino ethyl-methacrylate (DMAEM). (b) Dimethyl-amino ethyl-acrylate (DMAEA). (c) Diallyl-dimethyl ammonium chloride (DADMAC).

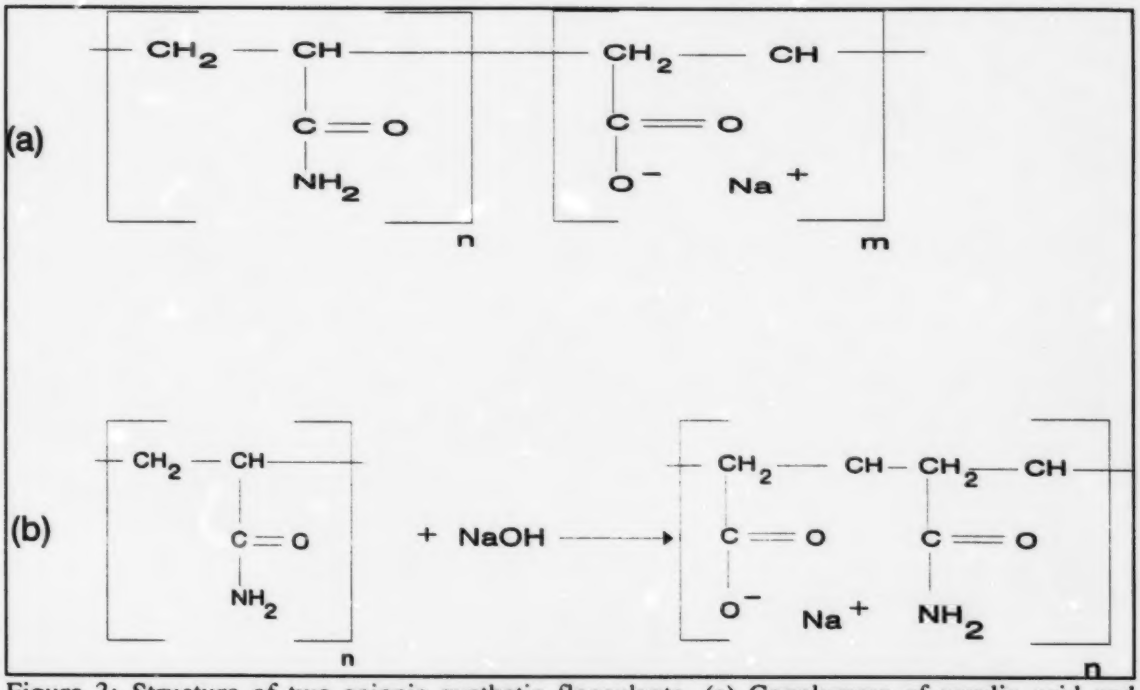

Figure 3: Structure of two anionic synthetic flocculants. (a) Copolymers of acrylic acid and acrylamide. (b) Hydrolysis of polyacrylamide.

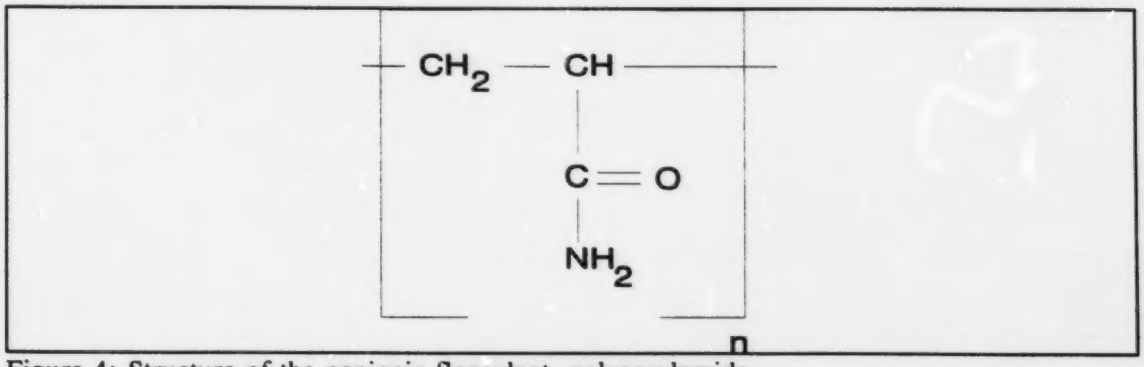

Figure 4: Structure of the nonionic flocculant, polyacrylamide.

CONCLUSIONS

The flocculating ability of microbial systems can be exploited in one of two ways. In the first the microbes are grown in the water that is to be treated; as the culture becomes

mature the microbes will flocculate and settle, bringing with them the suspended solids. This is the method that is presently being used in activated sludge treatment systems. The other method involves the growth of microbes for optimal polymer production; the polymers are extracted from the broth and then added to the waste water to be treated. The biopolymers are thus employed in a manner that is analogous to synthetic polymers.

Some positive results have been obtained by a number of researchers investigating biopolymers, basic knowledge concerning the chemical characteristics of these biopolymers is lacking and therefore hindering their widespread use in the treatment of waste waters for suspended solid removal.

RECOMMENDATIONS

In the experimental part of this work, the ability of polymers of microbial origin to increase the settling rates of clay suspensions will be compared to the performance of synthetic flocculants. A number of anionic and nonionic exopolysaccharides will be tested; in addition, biopolymers produced by a number of microorganisms that have not been characterized but have shown success with the flocculation of suspended solids will also be evaluated.

One of the goals of this work is to characterize the biopolymers in terms of ionic character and molecular weight so that comparisons can be made to the synthetic flocculants. This characterization will identify important properties for bioflocculants and thus will aid in the development of new bioflocculants. The biopolymers will be obtained in a purified form from chemical manufacturers when possible, otherwise they will be obtained from cultures of the source microorganism. The anionic biopolymers to be tested as well as its source microorganism are: alginate (Pseudomonas aeruginosa, Azobacter vinelandii), xanthan (Xanthomonas campestris), phosphomannan (Hansenula capsulata, H. hansenula) and a polysaccharide produced by Arthrobacter viscosus. Nonionics to be evaluated are: dextran (Acetobacter sp., Leuconostoc mesenteroides), pullulan (Pullularia pullulans), scleroglucan

(Sclerotium glucanicun) and curdlan (Alcaligenes faecalis). Other microorganisms that have been successful in flocculating suspended solids and will be tested are: black yeast (NRRL Y6272), Zoogloea ramigera and Rhodococcus erythropolis.

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